Platform F: Protein-Nucleic Acid Interactions I

62-Plat
Nucleic Acid Translocation By Hepatitis C Virus Helicase NS3h Is Dependent on Sugar and Base Moieties
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The NS3 helicase (NS3h) of hepatitis C virus (HCV) is a 3′ to 5′ SF2 RNA and DNA helicase that is essential for the replication of HCV. We have examined the kinetic mechanism of translocation of NS3h along single stranded nucleic acid with bases rU, dU and dT and found that the rate of translocation is dependent upon both base and sugar moieties. We find that the approximate rates of translocation are 3 nt/s (oligo-dT), 35 nt/s (oligo-dU), and 42 nt/s (oligo-rU). These macroscopic translocation rates correspond well to differences in the binding affinity of the translocating NS3h protein to the respective substrates. The values of K_M for NS3h translocating at a saturating ATP concentration are: 3.3 ($\pm$ 0.4) μM nucleotide (poly-dT), 27 ($\pm$ 2) μM nucleotide (poly-dU), and 36 ($\pm$ 2) μM nucleotide (poly-rU). Despite the differences in translocation rates and binding affinities, the ATP coupling stoichiometry for NS3h translocation is identical for all three substrates, with a value of ~2 nt per ATP consumed. The identical periodicity of ATP consumption implies a similar mechanism for NS3h translocation along each substrate. This data, together with our independently determined values of K_D for NS3h binding to poly-dT (220 $\pm$ 20 nM nucleotide) and poly-dU (430 $\pm$ 30 nM nucleotide), suggest that the differences in the macroscopic translocation rates may be explained by differences in the entropic contribution to the binding free energy of NS3h to the different nucleic acid substrates. This conclusion is consistent with observations from a previously published crystal structure of NS3h in complex with a short oligonucleotide (Kim, et al (1998) Structure 6:89-100).

63-Plat
Protein-mRNA Interactions Observed in Living Cells By Dual-Color Fluorescence Fluctuation Spectroscopy
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RNA binding protein controls the localization, translation and degradation of mRNA of specific proteins, therefore exerting a key influence on the cell metabolism, motility and differentiation. The zipcode binding protein 1 (ZBP1) binds to the 3′ untranslated region (UTR) of beta-actin mRNA and regulates its translation. Quantitative characterization of the interaction between mRNA and protein is crucial to dissect this regulation mechanism. We have developed a dual-color fluorescence fluctuation spectroscopy (FFS) technique to study the interaction between mRNA and protein directly in living cells. FFS determines the brightness, concentration and diffusion time of fluorescent particles from the intensity bursts generated by individual particles passing through a small observation volume. Dual-color FFS distinguishes fluorescent species by brightness and diffusion time as well as the fluorescence color. In this study, we apply dual-color FFS to study the interaction between beta-actin mRNA and ZBP1. The endogenous mouse beta-actin mRNA is visualized by incorporating 24 MS2 stem-loops in the 3′ UTR, which are specifically bound by fluorescently labeled MS2 coat protein (MCP). We have developed a tandem dimeric MCP system that is particularly suitable for FFS brightness analysis. Since multiple MCPs bind to a single mRNA, the brightness of mRNA is significantly higher than that of free MCP, which allows us to readily resolve mRNA from free MCPs. Furthermore, ZBP1 is labeled with a different color and dual-color FFS directly extracts the interacting mRNA-ZBP1 species. The interaction is visualized by brightness signatures and the binding affinity is extracted by measuring different cells with varying concentrations of ZBP1. This work is supported by National Institute of Health EB2060.

64-Plat
Hybridization Kinetics Is Different Inside Cells
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It is generally expected that the kinetics of reactions inside living cells differs from the situation in bulk solutions. Macromolecular crowding as well as specific binding interactions could change the diffusion properties and the availability of free molecules. Their impact on reaction kinetics in the relevant context of living cells is still elusive, mainly due to the difficulty of capturing fast kinetics *in vivo*. This paper shows spatially resolved measurements of DNA hybridization kinetics in single living cells. HeLa cells were transfected with a FRET labeled dsDNA probe by lipofection. We characterized the hybridization reaction kinetics with a kinetic range of 10 μs to 1 s by a combination of laser-driven temperature oscillations and stroboscopic fluorescence imaging. The time constant of the hybridization depended on DNA concentration within individual cells and between cells. A quantitative analysis of the concentration dependence revealed several-fold accelerated kinetics as compared to free solution for a 16 bp probe and decelerated kinetics for a 12 bp probe. We did not find significant effects of crowding agents on the hybridization kinetics *in vitro*. Our results suggest that the reaction rates *in vivo* are specifically modulated by binding interactions for the two probes, possibly triggered by their different lengths. In general, the presented imaging modality of TOOL (Temperature Oscillation Optical Lock-in) microscopy allows to probe biomolecular interactions in different cell compartments in living cells for systems biology.

65-Plat
Dynamics of the HIV Reverse Transcription Initiation Complex
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Reverse transcription of the HIV genome begins from a ternary complex containing a tRNA primer, the highly-structured viral RNA template, and the reverse transcriptase (RT) enzyme. Previous work on RT revealed that RT binds its nucleic acid substrates in a variety of different modes and dynamically switches between these modes, for example, flipping between different binding orientations and sliding between alternate positions on the substrate. To assess RT's dynamics on more complicated substrates and to understand how these dynamics influence the initiation of reverse transcription, we employ a single molecule FRET assay to monitor the interactions between RT and the initiation tRNA/viral RNA complex. These measurements define the binding configuration of RT at each stage of the extension of the tRNA primer. We find that RT can bind the initiation complex in two orientations, corresponding to a productive orientation and an inactive orientation, and spontaneously flip between the two orientations. Both the composition of the primer's 3′ end and the secondary structure of the template mediate RT's binding orientation. As RT extends the tRNA primer, the amount of time it spends in the productive binding orientation first decreases, then increases as more nucleotides are added to the end of the tRNA primer. These results mirror ensemble primer extension assays showing that RT acts slowly and distributively during the addition of the first few nucleotides, then transitions to a fast, processive mode. These results demonstrate that RT's binding dynamics regulate the initiation of reverse transcription and provide a mechanistic explanation for the changes in RT activity during initiation.

66-Plat
DNA Unwinding By DnaB and the DnaB/TAU Complex
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The replicative helicase for E. coli is DnaB, a hexameric, ring-shaped motor protein that encircles and translocates along ssDNA, denaturing dsDNA in advance of its motion by sterically occluding the complementary strand to the outside of the ring. Using multiplexed single-molecule measurements with magnetic tweezers, we investigate the translocation and unwinding activities of DnaB. We find that DnaB's interaction with the ss/dsDNA junction is dependent on the geometry of the DNA substrate and applied force, suggesting that the hexamer interacts with the occluded strand during unwinding. We have also found that the structure of the bound nucleotides within DnaB's central channel is highly compact relative to the contour length of ssDNA, consistent with crystal structures of related hexameric helicases. Finally, in all our experiments, we find high variance in the rates of unwinding as well as frequent pausing, indicating that individual hexamers fluctuate among different conformations with different activities. To investigate DnaB's variable nature, we test the effect on helicase activity of interactions with the tau subunit of the Pol III holoenzyme, which is thought to regulate DnaB's unwinding rate.

67-Plat
Chemo-Mechanical Study of a Hexameric Helicase on the Single-Molecule Level
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Cell division involves DNA replication that requires the opening of double stranded DNA by cellular machines known as helicases. Although many of